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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/627,556	LEDBETTER ET AL.	
	Examiner	Art Unit	
	LYNN BRISTOL	1643	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *8/14/08*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *See Continuation Sheet* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☒ Claim(s) *110* is/are allowed.

6)☒ Claim(s) *1-8,10,12,14-16,18-58,61,62,71,72,75,77-79,111 and 112* is/are rejected.

7)☒ Claim(s) *83,85,86,89-92,94-103,107 and 108* is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *10/13/08; 10/15/08*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

Continuation of Disposition of Claims: Claims pending in the application are 1-8,10,12,14-16,18-58,61,62,71,72,75,77-79,83,85,86,89-92,94-103,107,108 and 110-112.

DETAILED ACTION

1. Claims 1-8, 10, 12, 14-16, 18-58, 61, 62, 71, 72, 75, 77-79, 83, 85, 86, 89-92, 94-103, 107, 108 and 110-112 are all the pending claims for this application.

2. Claims 9, 11, 13, 17, 63-70, 73, 74, 76, 81, 82, 84, 87, 88, 93, 104-106, and 109 were cancelled and Claims 111 and 112 were added in the Response of 8/14/08.

3. Claims 1-8, 10, 12, 14-16, 18-58, 61, 62, 71, 72, 75, 77-79, 83, 85, 86, 89-92, 94-103, 107, 108 and 110-112 are all the pending claims under examination.

4. Applicants amendments to the claims have necessitated new grounds for rejection. This action is FINAL.

Information Disclosure Statement

5. The IDS' of 10/13/08 and 10/15/08 have been considered and entered with the exception of the copies of the Office Actions cited on the 1449 form filed with the IDS of 10/13/08. These Office Actions do not qualify as non-patent literature references within the meaning of 37 CFR 1.56. Accordingly, the Office Actions have been stricken on the form. Copies of the initialed forms are attached.

Withdrawal of Rejections

Claims - 35 USC § 112, second paragraph

6. The rejection of Claim 13 for the recitation "where leucine is replaced by des-leucine at position 11" is withdrawn and moot for the cancelled claim.

7. The rejection of Claim 110 for the recitation "G28-2VHL11S (SSC-P) H WCH2WCH3 set out in SEQ ID NO: 329" is withdrawn in view of the amendment of the claim to clarify the relationship of the terms.

Claims - 35 USC § 103

8. The rejection of Claims 1-12, 14-23, 25-28, 31-47, 52-55, 57, 58, 61-63, 65, 68, 69, 71, 72, 76-79 and 109 under 35 U.S.C. 103(a) as being unpatentable over Shan et al (J. Immunol. 162:6589-6595 (1999); hereinafter referred to as "Shan"; cited in the IDS of 7/2/04) in view of Pluckthun et al. (USPN 6,815,540; published 11/9/2004; filed 1/15/1999; hereinafter referred to as "Pluckthun") is withdrawn.

Applicants' amendment to Claims 1 and 77 by introducing the limitation for the hinge comprising a third cvsteine of a wild type IgG1 hinge region or having only the first and third cvsteines of a wild type IgG1 hinge region (Claim 1) or the hinge comprising a wild type IgG1 hinge region comprising first, second, and third cysteine residues, and a proline, wherein the first cysteine residue is N-terminal to the second cysteine, the second cysteine is N-terminal to the third cysteine, and the third cysteine is N-terminal to the proline residue and wherein the altered hinge region has only the third cysteine or has only the first and third cysteines of a wild type IgG1 hinge region (Claim 77) overcomes the rejection.

9. The rejection of Claims 1, 56, 65 and 70-72 under 35 U.S.C. 103(a) as being unpatentable over Shan in view of Pluckthun as applied to claim 1 above, and further in

view of Bodmer et al. (USPN 5,677,425; published 10/14/1997; hereinafter referred to as "Bodmer"; cited in the IDS of 12/22/04) is withdrawn.

See the examiner comments for withdrawing the rejection set forth above under section 8.

10. The rejection of Claims 1, 63, 66 and 82 under 35 U.S.C. 103(a) as being unpatentable over Shan in view of Pluckthun as applied to claims 1 and 77 above, and further in view of Bodmer and Morrison et al. (USPN 6,284,536; published 9/4/2001; filed 8/11/98; hereinafter referred to as "Morrison"; cited in the IDS of 3/21/05) is withdrawn.

See the examiner comments for withdrawing the rejection set forth above under section 8.

11. The rejection of Claims 1, 64, 67 and 73-75, 77 and 81 are rejected under 35 U.S.C. 103(a) as being unpatentable over Shan in view of Pluckthun as applied to claims 1 and 77 above, and further in view of Roux et al. (J. Immunol. 161:4083-4090 (1998); hereinafter referred to as "Roux") is withdrawn.

See the examiner comments for withdrawing the rejection set forth above under section 8.

Rejections Maintained

Claims - 35 USC § 112, first paragraph

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Enablement

12. The rejection of Claims 1-8, 10, 12, 14-16, 18-28, 31-58, 61, 62, 71, 72, 75, 77-79, 111 and 112 under 35 U.S.C. 112, first paragraph, is maintained because the specification and the prior art are not enabling for *any* scFv having antigen binding specificity for *any* antigen and comprising *any* amino acid substitution in any one or more positions 9, 10, 11, 12, 108, 110 and/or 112 for the VH region and *any* amino acid substitution in one or more of positions 12, 80, 81, 83, 105, 106 and/or 107 for the VL region.

a) For purposes of review, the original rejection was set forth in the Office Action of 12/8/06 as follows:

"a) The specification is not enabling for using single chain proteins having only a heavy chain domain (or only a light chain domain).

Claims 1, 3, 8, 25, 26, 33-52, 55-58, and 63-76 are broadly drawn to a binding domain polypeptide that has a heavy chain, which would not bind antigen because the molecule contains CDR1-3 from only one Ig chain. The specification does not enable a binding domain with only a heavy chain, but discloses single chain proteins and scFvs with at least one heavy chain variable domain and at least one light chain variable domain.

It is well established in the art that the formation of an intact antigen-binding site generally requires the association of the complete heavy and light chain variable regions of a given antibody, each of which consists of three CDRs which provide the majority of the contact residues for the binding of the antibody to its target epitope. The amino acid sequences and conformations of each of the heavy and light chain CDRs are critical in maintaining the antigen binding specificity and affinity which is characteristic of the parent immunoglobulin. It is expected that all of the heavy and light chain CDRs in their proper order and in the context of framework sequences which maintain their required conformation, are required in order to produce a protein having antigen-binding function and that proper association of heavy and light chain variable regions is required in order to form functional antigen binding sites. Even minor changes in the amino acid sequences of the heavy and light variable regions, particularly in the CDRs, may dramatically affect antigen-binding function as evidenced by Rudikoff et al (Proc Natl Acad Sci USA 1982 Vol 79 page 1979; cited in the IDS of March 21, 2005). Rudikoff et al. teach that the alteration of a single amino acid in the CDR of a phosphocholine-binding myeloma protein resulted in the loss of antigen-binding function. Thus, it is unlikely that scFv's as defined by the claims which may contain less than the full complement of CDRs from the heavy or light

chain variable regions or comprise any amino acid substitution in the hypervariable region of the heavy chain or light chain variable domain, have the required binding function. The specification provides no direction or guidance regarding how to produce single chain antibodies as broadly defined by the claims. Undue experimentation would be required to produce the invention commensurate with the scope of the claims from the written disclosure alone.

b) The specification is not enabling for any single chain antibody or scFv comprising any amino acid substitution or deletion in positions 9, 10, 11, 12, 108, 110 and 112 for the VH region and any amino acid substitution or deletion in one or more of positions 12, 80, 81, 83, 105, 106 and 107 for the VL region.

Claims 1-9, 14-79, and 81-109 are drawn to single chain proteins and scFv-Igs comprising any amino acid substitution or deletion in positions 9, 10, 11, 12, 108, 110 and 112 for the VH region and/or any amino acid substitution or deletion in one or more of positions 12, 80, 81, 83, 105, 106 and 107 for the VL region.

The specification does not enable single-chain protein molecules that are modified in protein sequences at positions 9, 10, 11, 12, 108, 110, or 112 for the heavy variable domain or positions 12, 80, 81, 83, 105, 106 and 107 for the light chain with just any amino acid insertion, deletion, substitution, etc. The specification supports the following amino acid substitutions at positions 9, 10, 11, 12, 108, 110, or 112 of the VH region and especially for position 11: serine, threonine, cysteine, tyrosine, asparagine, glutamine, aspartic acid, glutamic acid, lysinc, arginine, and histidine. The specification does not support the substitution of leucine at position 11 of the VH region with des-leucine.

The claims are not commensurate in scope with the enablement provided in the specification. The specification does not support the broad scope of the claims, which encompass all modifications to the amino acid sequence because the specification does not disclose the following:

The general tolerance to modification and extent of such tolerance;

The specific positions and regions of the sequence(s) which can be predictably modified and which regions are critical; and

The specification provides insufficient guidance as to which of the essentially infinite possible choices is likely to be successful.

Thus, applicants have not provided sufficient guidance to enable one of ordinary skill in the art to make and use the claimed protein in manner reasonable correlated with the scope of the claims broadly including any number of additions, deletions, or substitutions. The scope of the claims must bear a reasonable correlation with the scope of enablement. See In re Fisher, 166 USPQ 19 24 (CCPA 1970).

Without such guidance, the changes which can be made in the protein's structure and still maintain biological activity is unpredictable and the experimentation left to those skilled in the art is unnecessarily and improperly extensive and undue. See Amgen, Inc. v. Chugai Pharmaceutical Co. Ltd., 927 F,2d 1200, 18 USPQ 1016 (Fed. Cir. 1991) at 18 USPQ 1026 1027 and Ex parte Forman, 230 USPQ 546 (BPAI 1986).f

Further protein chemistry is probably one of the most unpredictable areas of biotechnology. For example, the replacement of a single lysine at position 118 of the acidic fibroblast growth factor by a glutamic acid led to a substantial loss of heparin binding, receptor binding, and biological activity of the protein (see Burgess et al, Journal of Cell Biology Vol 111 November 1990 2129-2138). In transforming growth factor alpha, replacement of aspartic acid at position 47 with asparagine, did not affect biological activity while the replacement with serine or glutamic acid sharply reduced the biological activity of the mitogen (see Lazar et al Molecular and Cellular Biology Mar 1988 Vol 8 No 3 1247-1252).

Replacement of the histidine at position 10 of the B-chain of human insulin with aspartic acid converts the molecule into a superagonist with 5 times the activity of nature human insulin. Schwartz et al, Proc Natl Acad Sci USA Vol 84:6408-6411 (1987). Removal of the amino terminal histidine of glucagon substantially decreases the ability of the molecule to bind to its receptor and activate adenylate cyclase. Lin et al Biochemistry USA Vol 14:1559-1563 (1975).

These references demonstrate that even a single amino acid substitution or what appears to be an inconsequential chemical modification, will often dramatically affect the biological activity of the protein.

c) The specification is not enabling for making hybridomas which express scFv or scFv-Ig fusion proteins comprising VH (or VL) regions being modified at any amino acid position.

Claims 29, 30 and 83-108 are interpreted as scFv-Igs having position 9, 10, 11, 12, 108, 110 and 112-modified binding domains (VH) being produced from hybridomas 2H7, HD37, G28-1, 4.4.220, Fc2-2, UCHL-1, 5B9, L6, 10A8, 2e12, 40.2.36, 1D8 or G19-4.

Applicant has not shown that any of these hybridomas express an antibody comprising a VH domain possessing the claimed modifications for positions 9, 10, 11, 12, 108, 110 and 112 (or a VL domain modified at positions 12, 80, 81, 83, 105, 106 and 107). Applicants have cloned the VH and VL domains from an antibody expressed by the respective hyrbidoma and genetically engineered amino acid modifications into the claimed positions for the VH and VL domains, but the specification does not disclose the generation of any recombinant hybridomas that would express a molecule for or comprising the claimed modified VH (or VL) domain much less an entire scFv molecule. It is generally accepted in the art that scFv are produced by recombinant techniques from an

idiotypic antibody expressed by a hybridoma (see Worn and Pluckthun (J. Mol. Biol. 305:989-1010 (2001) (describing strategies for producing recombinant scFVs with improved stability, especially Figure 4, listing CDR grafting, point mutation, etc. and methods for accomplishing these modifications using display libraries (see Figure 8)); Hollinger and Hudson (Nature Biotech. 23:1126-1136 (2005) (reporting preclinical and clinical studies for recombinant antibody fragments (Table 1) including engineering of IgGs into Fab, scFv an single variable VH and VL domains (p. 1127, Col. 1, ¶1).

The specification does not teach the method steps for producing the scFv-expressing recombinant hybridomas from the parent hybridoma cell lines. Thus one skilled in the art would be required to identify the appropriate constructs for transfecting cells if scFv encoded vectors were being used, selecting stable single-chain molecule expressing host cells under appropriate conditions, and screening supernantants for scFvs with specific binding activity. Alternatively, if Applicants contemplate using the parent hybridoma cell lines to produce the modified VH or VL, then one skilled in the art would be required to site-specifically mutagenize nucleic acids encoding the amino acid of interest to obtain the modified VH or VL, and the specification is silent with respect to accomplishing this endpoint.

Conclusion

Therefore, in view of the lack of guidance and lack of predictability associated with regard to making and using the single chain proteins and scFv-Ig fusion proteins encompassed in the scope of the claims, one skilled in the art would be forced into undue experimentation in order to practice the broadly claimed invention."

For purposes of review, the rejection was withdrawn for the claims reading on single variable domain (VH or VL) antibodies in the Office Action of 9/11/07 as follows:

"Applicants' admission on the record under section VI (a), pp. 18-19 of the Response of 6/8/07 "*that Claims 1 and 77 encompass a protein having at least a heavy chain variable region and a protein having both a heavy chain variable region and a light chain region"* and *"the claims do not recite a protein having only a light chain variable region"* overcomes the rejection for Claims 1-58, 61-79, and 81-109.

Applicants' discussion on p. 19 of the Response about the subset of functional antibodies having only a heavy chain variable region (e.g., camelid, sharks, etc.) is acknowledged but irrelevant in view of the admission of record above. Further, Applicants have not explained how the overall structures for the subset of single variable domain antibodies especially the hinge region is distinct and separate from the hinge of an IgG, for example, in the references cited in the Response (Nutall et al., Curr. Pharm. Biotechnol. 1:253-263 (2000); Muyldermans, J. Biotechnol. 74:277-302 (2001), Abstract). Nevertheless, Applicants admission of record that the claims are not drawn to camelid-like (or shark- or llama-like) antibodies renders the rejection moot.

Applicants' provide a single art reference (Ward et al., Nature 341:544-546 (1989); Abstract) describing a single domain antibody retaining antigen- binding affinity. Applicants have not demonstrated that this same method of producing a single domain antibody could be reliably, predictably and reproducibly practiced using just any variable domain from just any antibody. Applicants have not demonstrated the universality of producing a fully functional single domain antibody that meets all of the limitations of the instant claims. Nevertheless, Applicants admission of record that the claims are not drawn to single domain antibodies renders the rejection moot."

However, the rejection was maintained in part for the reasons set forth in the Office Action of 12/8/06 and 9/11/07 as follows:

"Applicants' allegations on pp. 20-21 have been considered but are not found persuasive. Applicants allege that because the specification teaches: VH substitution of position 11 with des-leucine (p. 20), [346] of the specification describes several references showing methods for engineering framework and CDR residues (p. 20), WO92/01787 and WO98/02462 describe residues of V domains in which substitutions can be made (p. 20), and methods for measuring functional properties of antibodies, the instant claims are fully enabled.

Applicants have not addressed any of the references cited in the Office Action to substantiate the Examiner's position that amino acid substitutions in proteins, more especially framework and CDR residues for antibodies, can dramatically effect or reduce the function of the antibody (see Rudikoff).

Applicants have not addressed the *predictability* prong of the WANDS test for making antibodies meeting all of the limitation of the claims. The skilled artisan would have been required to identify candidate VH and VL domains from any antibody recognizing any antigen, to have modified the amino acid residues in the recited positions of the VH and VL domains, and combined the modified VH and VL domains in order to have produced a single chain antibody or scFv capable of recognizing the antigen, having increased expression or stability, and having at least one immunological activity. At this point, one skilled in the art would need to have produced and expressed the antibodies, assessed binding activity against the parent antibody, and then finally performed any bioassays to measure antigen binding characteristics (e.g., binding specificity, equilibrium dissociation constant (K.sub.D), dissociation and association rates (K.sub.off and K.sub.on respectively), binding affinity and/or avidity). The technology to perform these experiments was available at the time of application filing, but one of ordinary skill in the art could not have predicted from the universe of single chain antibodies and scFv antibodies directed against the universe of antigens which one or combination of CDR and/or framework modifications in the VH and/or VL would generate a single chain antibody or scFv having antigen binding specificity for *any* antigen, increased expression or solubility *and* at least one immunological activity.

Therefore, in view of the lack of examples and lack of predictability associated with regard to producing the myriad single chain antibodies and scFvs which meet all of the claim limitations, one skilled in the art would be forced into undue experimentation in order to practice the broadly claimed invention."

Still further, the same rejection was maintained for the reasons set forth in the Office Action of 2/14/08 as follows:

"a) Applicants allege because the specification teaches: several references showing methods for engineering framework and CDR residues at [346], WO92/01787 and WO98/02462 which are incorporated by reference and describe residues of V domains in which substitutions can be made, Examples 20, 34, 35, 38, 41 and 42 demonstrate inventive scFv constructs, and methods for measuring functional properties of antibodies, the instant claims are fully enabled.

The examiner respectfully submits that the scope of all possible substitutions, deletions or combinations thereof for the designated residues of the VH and VL domains in the broadest claims could not be predicted by one of skill in the art to produce a mono- or bi-valent scFv with a) the same binding specificity as the parent antibody, b) have increased expression or stability over the parent antibody and c) be capable of at least one immunological activity.

Prior Art Status: CDR and framework interactions influence antigen recognition

At the time of Applicant's filing, the field of art acknowledged that amino acid composition influenced the conformation of CDR and framework interactions in variable domains which influenced binding and antigen recognition. Thus one could not predictably mutate an amino acid residue in an immunoglobulin variable domain and expect that the properties of antigen binding and recognition would not be affected.

McCalllum *et al.* (J. Mol. Biol. (1996) 262:732-745), analyzed many different antibodies for interactions with antigen and state that although CDR3 of the heavy and light chain dominate, a number of residues outside the standard CDR definitions make antigen contacts (see page 733, right col) and non-contacting residues within the CDRs coincide with residues as important in defining canonical backbone conformations (see page 735, left col.).

Pascalis *et al.* (The Journal of Immunology (2002) 169, 3076-3084) demonstrate that grafting of the CDRs into a human framework was performed by grafting CDR residues and maintaining framework residues that were deemed essential for preserving the structural integrity of the antigen binding site (see page 3079, right col.). Although abbreviated CDR residues were used in the constructs, some residues in all 6 CDRs were used for the constructs (see page 3080, left col.).

Casset *et al.* (2003) BBRC 307, 198-205 constructed a peptide mimetic of an anti-CD4 monoclonal antibody binding site by rational design and the peptide was designed with 27 residues formed by residues from 5 CDRs (see entire document). Casset *et al.* also states that although CDR H3 is at the center of most if not all antigen interactions, clearly other CDRs play an important role in the recognition process (page 199, left col.) and this is demonstrated in this work by using all CDRs except L2 and a framework residue located just before the H3 (see page 202, left col.).

Vajdos *et al.* (2002) J. Mol. Biol. 320, 415-428, additionally state that antigen binding is primarily mediated by the CDRs, and more highly conserved framework segments which connect the CDRs, are mainly involved in supporting the CDR loop conformations and in some cases framework residues also contact antigen (page 416, left col.).

Holm *et al* (2007) Mol. Immunol. 44: 1075-1084 describes the mapping of an anti-cytokeratin antibody where although residues in the CDR3 of the heavy chain were involved in antigen binding unexpectedly a residue in CDR2 of the light chain was also involved (abstract).

Chen *et al.* J. Mol. Bio. (1999) 293, 865-881 describe high affinity variant antibodies binding to VEGF wherein the results show that the antigen binding site is almost entirely composed of residues from heavy chain CDRs, CDR-H1, H2, H3 (page 866).

Wu *et al.* J. Mol. Biol. (1999) 294, 151-162 state that it is difficult to predict which framework residues serve a critical role in maintaining affinity and specificity due in part to the large conformational change in antibodies that accompany antigen binding (page 152 left col.) but certain residues have been identified as important for maintaining conformation.

Thus, while one can make the statement that the antibody or scFv can tolerate a limited number of changes at defined positions, the prior art recognizes that residues in both the CDRs and frameworks are shown to influence binding. In fact, the prior art as well as Applicants own disclosure do not support that it was clearly established, that CDR domains alone are sufficient to define the binding specificity of an antibody or scFv, and that multiple antibodies or scFv can predictably be generated having the same binding specificity based on predetermined residues in addition to having increased expression or stability and immunological activity.

Analyzing applicants own disclosure, which while it does have scFvs with divergent variable domains, the data seem to indicate that it is the frameworks and CDRs that contribute to antigen binding, increased expression and stability.

Prior Art Status: Conservative Amino Acid Substitutions within CDR/FR Residues

The claims encompass polypeptides comprising VH domains and VL domains comprising conservative amino acid substitutions. It is not well established in the art that all variable domains are amenable to conservative modifications. Numerous publications acknowledge that conservative substitutions would in fact change the binding ability of antibodies if not substantially reduce the affinity.

Brummell *et al.* (Biochemistry 32:1180-1187 (1993)) found that mutagenesis of the four HCDR3 contact residues for the carbohydrate antibody (Salmomella B O-polysaccharide) in no instance improved affinity but 60% of the mutants resulted in a 10-fold drop in binding constant (affinity electrophoresis value of 0.85), while still other mutants were lower (Table 1 and p. 1183, Col. 2, ¶2 to p. 1184, Col. 1, ¶1). Brummell demonstrates that no substitution retained antigen binding affinity similar to the wild type antibody despite targeted, conservative substitutions in known contact sites.

Kobayashi *et al.* (Protein Engineering 12:879-844 (1999)) discloses that a scFv for binding a DNA oligomer containing a (6-4) photoproduct with Phe or Tyr substitutions at Trp 33 retained "a large fraction of the wild-type binding affinity, while the Ala substitution diminished antigen binding" (Table 1). However, Kobayashi notes "replacing Trp 33 with Phe or Ala alters the local environment of the (6-4) photodimer since binding is accompanied by large fluorescence increases that are not seen with the wild-type scFv" (p. 883, Col. 2, ¶3).

Burks *et al.* (PNAS 94:412-417 (1997)) discloses scanning saturation mutagenesis of the anti-digoxin scFv (26-10) which also binds digitoxin and digoxigenin with high affinity and with 42-fold lower affinity to ouabain. 114 mutant scFvs were characterized for their affinities for digoxin, digitonin, digoxignenin and oubain. Histogram analysis of the mutants (Figure 2) reveals that "not all residues are optimized in even high affinity antibodies such as 26-10, and that the absence of close contact with the hapten confers higher plasticity, i.e., the ability to tolerate a wider range of substitutions without compromising binding (p. 415, Col. 2, ¶4- p. 416, ¶1).

Although Brummell *et al.*, Kobayashi *et al.* and Burks *et al.* introduced conservative amino acid substitutions into CDRs to examine binding effects these three references do not overcome the unpredictability in the art as far as demonstrating that any conservative substitution within any CDR can be made without affecting binding.

Jang *et al.* (Molec. Immunol. 35:1207-1217 (1998)) teach that single amino acid mutations to the CDRH3 of a scFV derived from 2C10, an anti-dsDNA autoantibody, reduced the binding activity about 20-50% compared to the unmutated scFv (Table 4).

Brorson *et al.* (J. Immunol. 163:6694-6701 (1999)) teach that single amino acid substitutions to the CDRs of IgM Abs for the bacterial protein, levan, are ablated.

Coleman (Research in Immunol. 145:33-36 (1994)) teaches that single amino acid changes within the interface of an antibody-antigen complex are important and that inasmuch as the interaction can tolerate amino acid sequence substitutions, "a very conservative substitution may abolish binding" while "in another, a non-conservative substitution may have very little effect on the binding" (p. 35, Col. 1, ¶1).

The specification provides insufficient guidance regarding how to produce the genus of antibodies or scFvs as broadly defined by the claims. Undue experimentation would be required to produce the invention commensurate with the scope of the claims from the written disclosure alone.

b) Applicants further allege the claims specify the amino acid positions which are to be changed and as such there are a limited number of choices to which a single amino acid can be altered, and because the residues in an

immunoglobulin variable domain are highly conserved, one of skill in the art can readily determine which residues may be altered without undue experimentation.

Despite Applicants urging the Office that claimed scFv constructs would not be unlimited in number, one must consider that for the broadest claims, a given amino acid position can be substituted with any one of the known 23 amino acids. The broadest claims are not limited to preferred amino acid substitutions. The broadest claims encompass scFvs comprising amino acid substitutions occurring in any one or more positions 9, 10, 11, 12, 108, 110 and 112 for the VH region and in any one or more of positions 12, 80, 81, 83, 105, 106 and 107 for the VL region thus a large number of possible combinations can be generated for the scFvs falling within scope of the claims.

Because the specification does not provide sufficient guidance for producing the myriad scFv constructs and the prior art recognizes the importance of amino acid interactions between the CDR and frameworks within variable regions for proper protein folding and antigen binding, the rejection of the claims for lack of enablement is maintained, and further where additional requirement of the invention is that the substitution, deletion or combination thereof confers increased expression and stability of the scFv."

b) Applicants allegations on pp. 15-18 of the Response of 8/14/08 have been considered and are not found persuasive. Applicants allege that because only a limited number of amino acid positions substituted for the VH and VL domain of any given scFv and because the specification and prior all teach methods of engineering framework and CDR regions in the variable domain, and methods for achieving functional antibodies, the experimentation to produce the scope of scFvs would not be burdensome. Further because the residues in a variable region are highly conserved (referencing Kabat), the ordinary artisan could determine which residues are conserved in the binding domain.

Response to Arguments

The examiner emphasizes and re-iterates the claims are interpreted as encompassing any scFv known or yet to be discovered having the substitution modified VH and VL regions at positions 9, 10, 11, 12, 108, 110 and 112 for the VH region and positions 12, 80, 81, 83, 105, 106 and 107 for the VL region. Applicants attempt to simplify this unpredictable technology by arguing that introduction of only a few amino substitutions to a VH or VL region would fall within ordinary experimentation of the

skilled artisan, yet at the same time, wholly ignore the teachings of all of the art references cited in the Office Actions of 12/8/06 and 2/14/08 for the general unpredictability of modifying VH and VL domains from any scFv.

These references were cited as showing the art recognized even conservative amino acid substitutions in a CDR could not predictably generate a functional binding antibody irrespective of the antibody/antigen specificity. At the very least Coleman is a reference that makes a generic disclosure that single amino acid changes within the interface of an antibody-antigen complex are important and that inasmuch as the interaction can tolerate amino acid sequence substitutions, "a very conservative substitution may abolish binding" while "in another, a non-conservative substitution may have very little effect on the binding." Coleman at the very least emphasizes the unpredictability for modifying antibody CDR domains. Further, the claims are not even limited to a conservative substitution but encompass any substitution so long as the substitution occurs at positions 9, 10, 11, 12, 108, 110 and/or 112 for the VH region and positions 12, 80, 81, 83, 105, 106 and/or 107 for the VL region.

Applicants have not identified or claimed what the consensus sequences for each CDR and each framework are for the instant claimed scFvs that would permit consistent, predictable and accurate production of any substituted scFv having specific binding and at least one immunological activity. None of these consensus sequences or conservative amino acid modifications is introduced as limitations into the claims hence the claims read on numerous unpredictable embodiments for myriad scFvs. The features upon which applicant relies (i.e., the consensus sequences for the VH and VL

domains for the universe of antibodies extrapolated from Kabat) are not recited in the rejected claim(s). Although the claims are interpreted in light of the specification, limitations from the specification are not read into the claims. See *In re Van Geuns*, 988 F.2d 1181, 26 USPQ2d 1057 (Fed. Cir. 1993). The instant claims are not enabled because the structure is not correlated with a functional relationship between the substituted VH and VL for the myriad scFvs and the ability to bind the myriad corresponding antigens and possess an immunological activity.

For all the reasons set forth above and in the Office Actions of 12/8/06, 9/11/07 and 2/14/08, the rejection is maintained.

New Grounds for Rejection

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

13. Claims 18, 19 and 77-79 rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

a) Claims 18 and 19 are indefinite for the recitation "wherein said light chain variable region has *one additional* amino acid substitution" because none of the preceding claims require the light chain variable region to have a substitution in the first instance so that the substitutions in Claims 18 and 19 are additional to a pre-existing substitution(s).

b) Claims 77-79 are indefinite for the recitation in Claim 77 "wherein the wild type IgG1 hinge region comprises first, second, and third cysteine residues, and a proline" because later in Claim 77 it recites "wherein the altered hinge region has only the third cysteine or has only the first and third cysteines of a wild type IgG1 hinge region." The first limitation is interpreted as all four of the amino acids being required in the hinge peptide whereas the second limitation is interpreted as there being less than all four amino acids present. Clarification is requested.

Priority

14. The later-filed application must be an application for a patent for an invention which is also disclosed in the prior application (the parent or original nonprovisional application or provisional application). The disclosure of the invention in the parent application and in the later-filed application must be sufficient to comply with the requirements of the first paragraph of 35 U.S.C. 112. See *Transco Products, Inc. v. Performance Contracting, Inc.*, 38 F.3d 551, 32 USPQ2d 1077 (Fed. Cir. 1994).

The disclosure of the prior-filed application, Application No. 10/053,530, filed 1/17/02 (abandoned) and Provisional Application No. 60/367,358 (converted from Application No. 09/765,208), filed 1/17/01, fail to provide adequate support or enablement in the manner provided by the first paragraph of 35 U.S.C. 112 for one or more claims of this application. The '358 and '530 applications disclose scFv-Ig, scFv-ligand and scFv-Fc fusion proteins where the VH and VL regions for the scFv are cloned from the 2H7, HD37, L6 and G28-1 hybridomas. The '358 and '530 applications

do not support modifications to VH or VL regions at any of amino acid positions 9, 10, 11, 12, 108, 110 and 112 of the heavy chain variable region and one or more amino acids at positions 12, 80, 81, 83, 105, 106 and 107 of the light chain variable region. Furthermore, the '358 and '530 applications do not contemplate making scFv proteins from any one of the Mabs, 4.4.220, Fc2-2, UCHL-1, 5B9, 10A8, 2e12, 40.2.36, G19-4 or 1D8.

The disclosure of '530 and '358, fail to provide adequate support or enablement in the manner provided by the first paragraph of 35 U.S.C. 112 for the limitations for the instant claimed hinges of Claim 1 (CXC or XXC).

The claims receive no benefit of the priority dates and for purposes of applying prior art, the claims are accorded the filing date for the instant application, 7/26/2003.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

The factual inquiries set forth in *Graham* **v.** *John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.

4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

15. This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 1-8, 10, 12, 14-16, 18-28, 31-58, 61, 62, 71, 72, 75, 111 and 112 are rejected under 35 U.S.C. 103(a) as being obvious over Shan et al (J. Immunol. 162:6589-6595 (1999); hereinafter referred to as "Shan"; cited in the IDS of 7/2/04) in view of Pluckthun et al. (USPN 6,815,540; published 11/9/2004; filed 1/15/1999; hereinafter referred to as "Pluckthun"; cited in the PTO 892 form of 12/8/06) and Ledbetter et al. (US 20030118592 (10/207,655); published June 26, 2003; filed July 25, 2002; hereinafter referred to as "Ledbetter").

The applied reference (US 20030118592 (10/207,655)) has common inventors with the instant application. Based upon the earlier effective U.S. filing date of the reference, it constitutes prior art only under 35 U.S.C. 102(e). This rejection under 35 U.S.C. 103(a) might be overcome by: (1) a showing under 37 CFR 1.132 that any invention disclosed but not claimed in the reference was derived from the inventor of

this application and is thus not an invention “by another”; (2) a showing of a date of invention for the claimed subject matter of the application which corresponds to subject matter disclosed but not claimed in the reference, prior to the effective U.S. filing date of the reference under 37 CFR 1.131; or (3) an oath or declaration under 37 CFR 1.130 stating that the application and reference are currently owned by the same party and that the inventor named in the application is the prior inventor under 35 U.S.C. 104, together with a terminal disclaimer in accordance with 37 CFR 1.321(c). This rejection might also be overcome by showing that the reference is disqualified under 35 U.S.C. 103(c) as prior art in a rejection under 35 U.S.C. 103(a). See MPEP § 706.02(l)(1) and § 706.02(l)(2).

The interpretation of Claims 1-8, 10, 12, 14-16, 18-28, 31-58, 61, 62, 71, 72, 75, 111 and 112 is of record from the Office Actions of 12/8/06, 9/11/07 and 2/14/08. Claim 1 has been amended to replace "the connecting region" with an altered wild-type IgG hinge region comprising “only the third cysteine” or “the first and third cysteines” of element ii). *Claims 77-79 are not included under this rejection because of the indefiniteness of the hinge region as discussed under section 13 above, but could be joined upon amendment.*

The claimed single chain proteins were prima facie obvious at the time of the invention over Shan, Pluckthun and Ledbetter.

Shan teach a scFv that binds CD20 that has the linker Gly-Gly-Gly-Gly-Ser or $(Gly\text{-}Gly\text{-}Gly\text{-}Gly\text{-}Ser)_3$, where the scFv is fused to a hinge that has the cysteines removed so it cannot dimerize and the hinge is fused to a CH2 and CH3 (human IgG1

Fc domain). Since the fusion protein has the hinge, CH2 and CH3, it is inherent that the protein has complement fixation or antibody-dependent cell-mediated cytotoxic properties, which further involve biological signaling, effector cell function, cellular activation and differentiation, release of biological molecules, and would also result in neutralization of infectious agent or toxins. Shan discloses the scFv-Ig molecule having apoptosis-inducing activity for a CD20-expressing tumor cell line. Since the molecule induces apoptosis, it is inherent that the protein induces activation of cellular signals, and up- and down-regulation of transcription. Shan teaches that the scFv-Ig constructs are useful for targeted immunotherapy of CD20-expressing B-cell malignancies including applications involving radionucleide conjugates and pretargeting strategies using scFv-streptavidin conjugates in combination with biotin-^{90}Y secondary reagents. Since the molecule has other targeting properties, it is inherent that the protein can bind to an intracellular target to affect a function. Shan does not teach introducing amino acid substitutions into the VH or VL regions of the scFv, that residues 9, 10, 11, 108, 110 and 112 of the HV and residues 12, 80, 81, 83, 105, 106 and 107 of the VL are substitutable, that the modifications confer increased production vis-à-vis increased stability, and that the modified scFvs can be fused to toxins, bacterial or viral-derived molecules or cytokines for further bio-affecting properties. Pluckthun rectifies these deficiencies in its disclosure.

Pluckthun discloses introducing into the VH and VL regions, combinations of amino acid substitutions, insertions and deletions effected to reduce the hydrophobicity, or which otherwise increase the solubility and levels of expression for the antibody

molecules and fragments. Pluckthun discloses the amino acid(s) which replace(s) the more hydrophobic amino acids include Asn, Asp, Arg, Gln, Glu, Gly, His, Lys, Ser, and Thr. Pluckthun discloses that residues 9, 10, 12, 15, 39, 40, 41, 80, 81, 83, 103, 105, 106, 106A, 107, 108 for VL, and residues 9, 10, 11, 13, 14, 41, 42, 43, 84, 87, 69, 105, 108, 110, 112, 113 for VH, are preferred positions for modifications. Pluckthun discloses VH- and VL-modified antibody molecules and fragments being linked by the $(Gly4Ser)_3$ peptide and further fused to other moieties which have a useful therapeutic function such as bacterial toxins Pseudomonas exotoxin A, and diphtheria toxin, virus surface molecules, plant toxins ricin, abrin, modeccin, saporin, and gelonin, and a cytokine, such as IL-2. Pluckthun discloses an example of a scFv mutant at VH position 11 (Flu6 (L11D/V84D) (FIG. 3B lane 7, 8) that yielded about 0.25 mg per liter of protein whereas the wt scFv antibody did not give any soluble protein. Because the molecules comprise other effector molecules, it is inherent that protein as a whole has cell signaling properties, viral, bacterial and toxin neutralizing properties, and immune cell inducing properties.

Both Shan and Pluckthun appreciate the advantages of producing smaller antibody fragments in order to reduce immunogenicity and allow for greater penetration into targeted sites. Applicants own dependent claims 4 and 5 are drawn to bivalent antibodies so Claim 1 would encompass mono- and bivalent antibodies. Improvements to bivalent antibodies and monovalent antibodies were taught by Shan in combination with Pluckthun. The motivation to produce a small sized scFv antibody having improved expression or stability would have been provided by Pluckthun. Pluckthun may suggest

that substitution of position 11 was not as effective as substituting other positions, but Pluckthun's effect at position 11 was observable. The instant generic claims are not limited by any amount to which the increased expression or stability of the protein must be achieved for the substitution or deletion of position 11 in the VH domain relative to the parent antibody, and yet still retain antigen binding and possess at least one immunological activity.

Neither Shan nor Pluckthun teach or suggest introducing an IgG hinge region into the polypeptide structure where the hinge comprises an altered wild-type IgG hinge region comprising "only the third cysteine" or "the first and third cysteines". Neither Shan nor Pluckthun teach the polypeptide structure derived from the antibodies HD37, 2H7, G28-1, 5B9, 10A8, 2e12, L6 and 1D8. Ledbetter rectifies these deficiencies.

Ledbetter teaches a binding domain-immunoglobulin fusion protein, comprising a binding domain polypeptide fused to a mutated human IgG1 hinge region comprising one cysteine residue (XXC) or two cysteine residues (SSC) in order to reduce dimerization, where the hinge region is joined to an immunoglobulin heavy chain CH2 constant region and heavy chain CH3 constant region polypeptide, and wherein said binding domain-immunoglobulin fusion protein specifically binds to an antigen and promotes antibody dependent cell-mediated cytotoxicity or complement fixation or both, wherein the binding domain polypeptide comprises a light chain variable region and a heavy chain variable region comprising amino acid substitutions. Ledbetter teaches mutation of leucine to serine at position 11 in the first framework region of the heavy chain variable region.

It would have been prima facie obvious to the ordinary artisan at the time the invention was made to have created a scFv-Ig fusion molecule as taught by Shan, Pluckthun and Ledbetter who teach introducing amino acid substitutions in the VH and VL of antibody fragments from the disclosed parent antibodies and linking the antibody fragments to Fc domains thru a non-dimerizing, mutated human IgG1 hinge in order to obtain more stable and increased production of the protein molecules.

One of ordinary skill in the art would have been motivated to and had a reasonable expectation of success to produced the instant the molecule based on the disclosure of Shan, Pluckthun and Ledbetter because Shan teaches in general that the scFv-Ig constructs are useful for targeted immunotherapy of malignancies including applications involving secondary reagents, and importantly, that the constructs are amenable to further structural modifications (p. 6954, Col. 2). One skilled in the art would have been motivated to have introduced protein stabilizing and protein-expression enhancing modifications into VH and VL regions of the scFv-Ig of Shan based on the teachings of Pluckthun and Ledbetter, because Pluckthun and Ledbtter teach that antibody fragments can be obtained in improved yields compared to wild-type molecules with the introduction of position-critical amino acid substitutions in the Vh, VL and hinge regions that specifically affect protein yield.

One skilled in the art would have had a reasonable expectation of success in producing the instant claimed scFv-Igs based on the combined disclosures because Pluckthun and Ledbetter disclose which positions are critical and what amino acids can be substituted for these positions in the scFv-Ig antibodies disclosed in the references

and because the binding domains according to Shan are scFv comprising VH and VL domains. One skilled in the art would have had a reasonable expectation of success in producing the instant claimed scFv-Igs based on the combined disclosures of Shan, Pluckthun and Ledbetter because Pluckthun and Ledbetter disclose that increased stability and protein yields are not compromised when scFvs are fused to other bio-affecting molecules and can be improved when cysteine residues are nodified in the hinge region to reduce dimerization. Thus further modifications of the scFv as taught by Shan, e.g., Ig fusion or bio-affecting proteins, would not have compromised the intended effect of introducing amino acid modifications into the VH and VL and hinge for increasing yield.

Thus the instant claimed invention was prima facie obvious at the time the invention was made, based on the combined reference disclosures.

Conclusion

16. Claims 110 is allowed.

17. Claims 83, 85, 86, 89-92, 94-103, 107 and 108 are objected to as being dependent upon a rejected base claim, but would be allowable if rewritten in independent form including all of the limitations of the base claim and any intervening claims.

18. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Lynn Bristol whose telephone number is 571-272-6883. The examiner can normally be reached on 8:00-4:00, Monday through Friday.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Larry Helms can be reached on 571-272-0832. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

LAB

/David J Blanchard/
Primary Examiner, Art Unit 1643